

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
9 October 2003 (09.10.2003)

PCT

(10) International Publication Number  
**WO 03/082794 A1**

(51) International Patent Classification<sup>7</sup>: C07C 65/40,  
A61K 31/192

(21) International Application Number: PCT/IN03/00073

(22) International Filing Date: 24 March 2003 (24.03.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
10/107,806 28 March 2002 (28.03.2002) US

(71) Applicant (for all designated States except US): COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH [IN/IN]; Rafi Marg, New Delhi 110 001 (IN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SHIVANAN-DAPPA, Thimmappa [IN/IN]; Central Food and Technological Research Institute, Mysore, Karnataka 570 013 (IN). SATTUR, Avinash, Prahalad [IN/IN]; Central Food and Technological Research Institute, Mysore, Karnataka 570 013 (IN). SHEREEN [IN/IN]; Central Food and Technological Research Institute, Mysore, Karnataka 570 013 (IN). DIVAKAR, Soundar [IN/IN]; Central Food and Technological Research Institute, Mysore, Karnataka 570 013 (IN). KARANTH, Nayalana, Kotte, Ganesh [IN/IN]; Central Food and Technological Research Institute, Mysore, Karnataka 570 013 (IN).

(74) Agent: SRINIVASAN, Thiagarajan; Kumaran & Sagar, 84-C, C6 Lane, Off Central Avenue, Sainik Farms, New Delhi 110 062 (IN).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declaration under Rule 4.17:**

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

**Published:**

— with international search report  
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A COMPOUND AS CHOLINESTERASE INHIBITOR AND ITS ISOLATION FROM FUNGUS *SPOROTRICHUM* SPECIES

(57) Abstract: The present invention provides a novel bioactive compound 12- (2'-CARBOXY- 5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE "Sporotricolone", mainly as acetylcholinesterase (AchE) inhibitor, along with a process for the isolation of said compound from fungus *Sporotrichum* species.

WO 03/082794 A1

## A COMPOUND AS CHOLINESTERASE INHIBITOR AND ITS ISOLATION FROM FUNGUS *SPOROTRICHUM* SPECIES

### TECHNICAL FIELD

5 The present invention relates to a compound 12- (2'-CARBOXY- 5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE "Sporotricolone", mainly as acetylcholinesterase (AChE) inhibitor. The present invention also relates to a process for the isolation of said compound from fungus *Sporotrichum* species.

### BACKGROUND ART

10 Enzyme inhibitors are important class of molecules that are used as drugs and pesticides. The enzyme acetylcholinesterase (AChE) is involved in the synaptic transmission of the nerve impulse and its inhibition leads to accumulation of the neurotransmitter, acetylcholine leading to overexcitation of the postsynaptic neuron. This property of the inhibitor has been exploited to develop newer insecticides against a wide range of insect  
15 pests as well as drugs effective against worms, and, recently a new class of neuroactive drugs against dementia (Alzheimer's).

Although earlier authors have isolated metabolites such as asteric acids, questin and questinol from *Sporotrichum* sp., no AChE inhibitor activity has been reported (Slater, GP, Haskins, RH and Hogge, L.R. Can J Microbiol 17 (1971), 1576-79). The fungi, *Aspergillus*  
20 *terreus* (Ling, KH, Liou, HH, Yang, CM and Yang CK, Appl. Env. Microbiol, 37 (1979) 355-57) and *Penicillium* sp. (Omura, Skuno, F, Otaguro, K, Shiomi, K. Mauma, R and Iwai, Y. J. Antibiot. 48(1995) 745-46 ) have been reported to produce an AChE inhibitor named Arusigacin. However, the AChE inhibitor of the present invention is isolated from *Sporotrichum* having distinct chemical structure and properties and therefore a novel  
25 inhibitor molecule.

After screening of various microorganisms, a fungal culture is selected which shows inhibition against a serine esterase/ protease/ cholinesterase enzyme. This imperfect deuteromycetes, *Sporotrichum* species and was first isolated in 1966. The taxonomic features of *Sporotrichum* species (deuteromyces) are broad hyphae and septate in nature;  
30 has hyaline conidiophores with little differentiation from vegetative hyphae and solitary conidia with broad attachment to the hyphae.

This culture has previously been a subject of research investigation at the Central Food Technological Research Institute (CFTRI) India, for its ability to grow on lignocellulosic

wastes for the production of enzymes and organic acids (Sreekantaiah, KR, PhD thesis (1976) University of Mysore; Manonmani, HK, PhD thesis (1986) University of Mysore).

This culture has now been used in the present invention to produce a fermented extract containing a serine esterase / protease/ cholinesterase inhibitor.

- 5 The conditions of fermentation have been described earlier in Indian Patent Application No. 303/DEL/2000 Sattur, AP, Shivanandappa, T, Divakar, S and Karanth, NG.

### OBJECTS OF THE INVENTION

The main object of the present invention is to provide bioactive compound 12- (2'-CARBOXY-5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE

- 10 "Sporotricolone" having inhibitory activity against acetylcholinesterase (AChE).

Another object of the present invention is to provide a compound having inhibitory activity against seiren esterase and protease.

Yet another object of the present invention is to provide a compound having insecticidal properties.

- 15 Still another object of the present invention is to provide a compound having enhanced cholinergic activity.

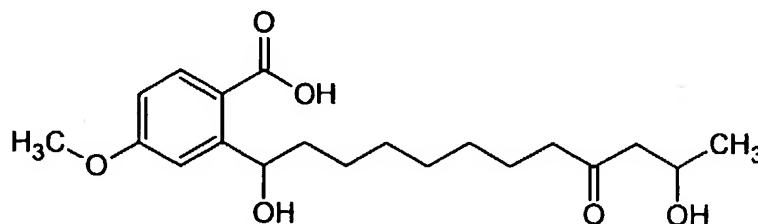
Yet another object of the present invention is to provide a process for the isolation of the above said compound.

### SUMMARY OF THE INVENTION

- 20 The present invention provides a compound 12- (2'-CARBOXY- 5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE "Sporotricolone" mainly as acetylcholinesterase (AChE) inhibitor. The present invention also provides a process for the isolation of said compound from fungus *Sporotrichum* species.

### DETAILED DESCRIPTION OF THE INVENTION

- 25 Accordingly, the present invention provides a bioactive compound 12- (2'-CARBOXY-5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE "Sporotricolone" of formula (I) having acetylcholinesterase (AChE) enzyme inhibition activity obtained from fungus *Sporotrichum* species.



FORMULA (I)

An embodiment of the present invention wherein the compound 12- (2'-CARBOXY- 5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE "Sporotricolone" of formula (I) having the following characteristic properties:

**Solubility:** Highly soluble in ethyl acetate, methanol and acetone.

5 **UV (ethyl acetate)  $\lambda_{\max}$ :** 265 nm, 312 nm.

**$^1\text{H}$ NMR spectrum (DMSO,  $\delta_{\text{TMS}} = 0.00$  ppm);**

$\delta$  1.03 (3H, d,  $J=6.3\text{Hz}$ ,  $-\text{CH}-\text{CH}_3$ )

$\delta$  1.2-2.7 (14H, m,  $7\times -\text{CH}_2$ )

$\delta$  3.6-3.8 (m,  $\text{Ar}-\text{O}-\text{CH}_3$  and  $\text{Ar}-\text{CH}-\text{OH}$ )

10  $\delta$  7.20 (1H, d,  $J=2.5\text{Hz}$ ,  $\text{C}_6-\text{H}$ )

$\delta$  7.30 (2H, d,  $J = 7.1 \text{ Hz}$ ,  $\text{C}_3'-\text{H}$  and  $\text{C}_4'-\text{H}$ )

**Mass spectrum (EI, 70 eV,  $25^\circ\text{C}$ , 200-ul amp):**

$m/e$ : 336 ( $\text{M}^+$ ), 279(366-87), 167(274-112), 57 ( $\text{CH}_2\text{COCH}_3$ ), 43, 29.

15 Another embodiment of the present invention, wherein the purity of the compound is established by TLC and RP HPLC.

Yet another embodiment of the present invention, wherein said compound is named as 12-(2'-CARBOXY- 5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE "Sporotricolone".

20 Still another embodiment of the present invention, wherein said compound is an inhibitor of the enzyme acetylcholinesterase from the rat brain as well as erythrocytes with a  $\text{IC}_{50}$  value of  $20 \times 10^{-6} \text{ M}$ .

Yet another embodiment of the present invention, wherein said compound also acts as an inhibitor of serine esterase of the rat liver serum.

25 Still another embodiment of the present invention, wherein said compound having insecticidal properties.

Yet another embodiment of the present invention, wherein said compound effective against mosquito larvae at an optimum concentration of  $70 \mu\text{g/ml}$  water (70 ppm) when exposed for 24 hrs.

30 Still another embodiment of the present invention, wherein the insecticidal activity of the compound against mosquito larvae is selected from *culex quinquefasciatus*.

Yet another embodiment of the present invention, wherein said compound as acetylcholinesterase inhibitor having potential application as a drug for Alzheimer's disease or dermentia.

The present invention also provides a process for the isolation of 12- (2'-CARBOXY- 5'-METHOXYPHENYL)-2,12-DIHYDROXY-DODECA-4-ONE Sporotricolone from the fungus Sporotrichum species, said process comprising the steps of:

- (a) extracting the fermented solid with an organic solvent;
- 5 (b) filtering the extract of step (a) through a cloth or Whatman filter paper to obtain a clear solution;
- (c) evaporating the solution of step (b) under reduced pressure to obtain a crude extract;
- (d) purifying the crude extract of step (c) by column chromatography over silica gel  
10 and eluting with mixture of organic solvents of increasing polarity;
- (e) pooling active eluted fraction of step (d) and further subjected to column chromatography over silica gel by eluting with mixture of organic solvents with increasing polarity;
- (f) repooling the active eluted fractions of step (e);
- 15 (g) evaporating the pooled fractions of step (f) to get a residue; and
- (h) dissolving the residue in step (g) in ethyl acetate to yield the pure compound "Sporotricolone".

Yet another embodiment of the present invention, a process wherein in step (a) the organic solvent is selected from a group consisting of ethyl acetate, acetone or methanol and  
20 preferably ethyl acetate.

Still another embodiment of the present invention, a process wherein in step (d) the mixture of organic solvents is selected from the combination of hexane: diethyl ether and chloroform: methanol mixtures.

Further embodiment of the present invention, a process wherein in step (e) the mixture of  
25 organic solvent used is chloroform: ethyl acetate mixture.

Yet another embodiment of the present invention, wherein the said compound is separated and purified by column chromatography on silica gel and RP HPLC.

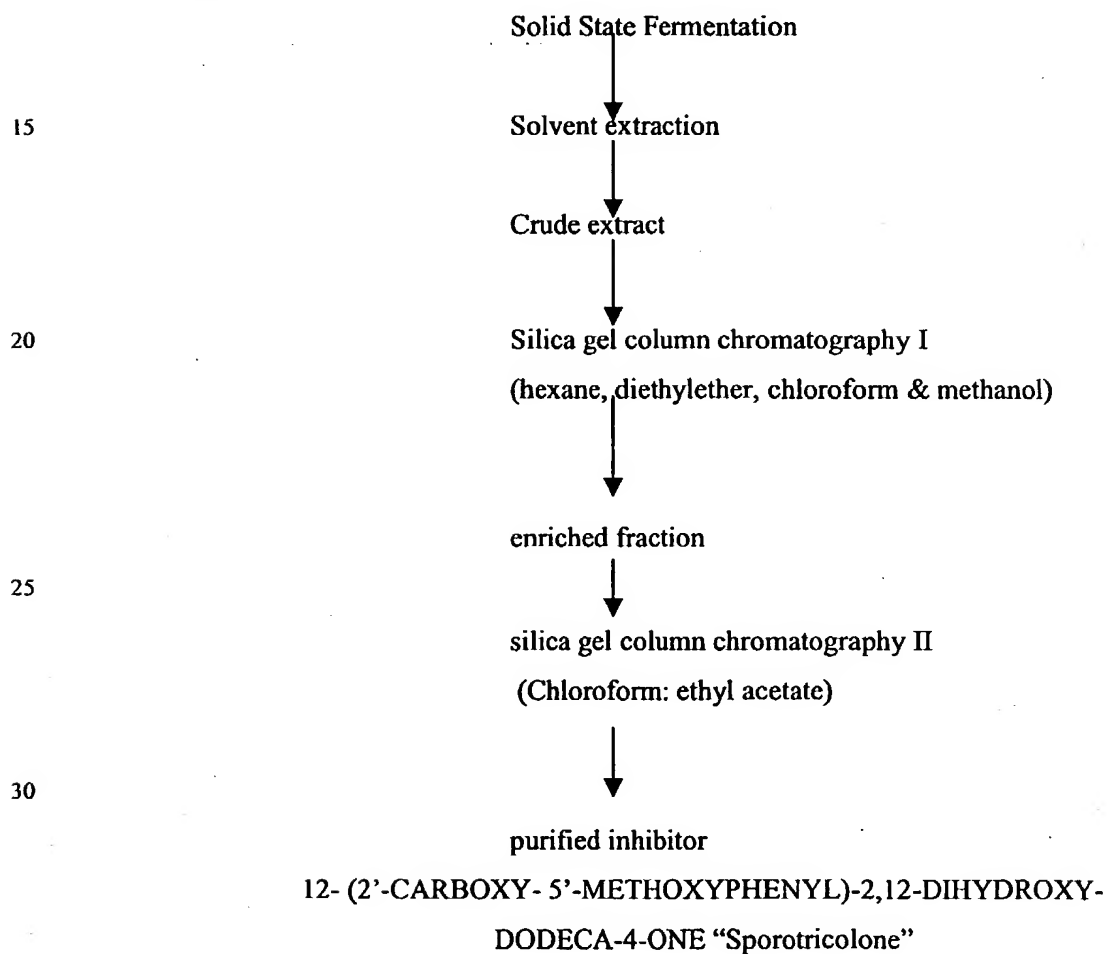
Still another embodiment of the present invention, wherein said compound having an UV absorption at 265 and 312 nm.

30 The present invention is further explained in the form of following embodiments

In the present invention a process for the isolation of an acetylcholinesterase inhibitor, which comprises the extraction of the fermented broth culture with solvents such as ethyl acetate. The crude extract is further extracted with 10-20 ml of methanol and subjected to column chromatography using silica gel and eluted with various combinations of solvents

such as hexane: diethyl ether (85:15, 50:50) followed by chloroform: methanol (95:5, 50:50, 10:90). Fractions are evaporated under nitrogen, dissolved in ethyl acetate and assayed for acetylcholinesterase (AChE) inhibition. The active fractions are pooled and further subjected to purification on silica gel column chromatography and eluted with chloroform: ethyl acetate (90:10, 50:50, 0:100). The active fractions pooled and the solvent evaporated and dissolved in 2 ml ethyl acetate. The purity, as checked by TLC, showed a single spot and HPLC on reverse phase column (C18) with chloroform and methanol as mobile phase. The yield is about 10 mg. The purified inhibitor showed inhibitor potency against rat brain AChE with an  $IC_{50}$  of  $15-20 \times 10^{-6}$  M.

A general process for the production of the novel Acetylcholinesterase inhibitor is given in the flow sheet:



The structure of the isolated inhibitor is determined by UV,  $^1H$  NMR and mass spectrometry.

The following examples are given by way of illustration of the present invention and should not be construed to limit the scope of the invention.

#### EXAMPLE – 1

The fermentation culture is extracted with 100 ml of ethyl acetate, filtered through a cotton  
5 filter and concentrated in vacuo to obtain 1 ml of the crude extract. This is used as a source of the enzyme inhibitor and 20  $\mu$ l of the extract gave 60-90 % inhibition of rat brain acetylcholinesterase enzyme.

#### EXAMPLE -2

The crude extract is further extracted with 10-20 ml of methanol and subjected to column  
10 chromatography using silica gel and eluted with various combinations of solvents such as hexane: diethyl ether (85:15, 50:50) followed by chloroform: methanol (95:5, 50:50, 10:90). Fractions are evaporated under nitrogen, dissolved in ethyl acetate and assayed for acetyl cholinesterase (AChE) inhibitor. The active fractions (# 11-19) are pooled and further subjected to purification on silica gel column chromatography and eluted with  
15 chloroform: ethyl acetate (90:10, 50:50, 0:100). The active fractions pooled and the solvent evaporated and dissolved in 2 ml ethyl acetate. The purity, as checked by TLC, showed a single spot. RP HPLC also ascertained the purity on a C18 column with chloroform and methanol as the mobile phase wherein it is a single peak. The yield is about 10 mg.

#### EXAMPLE – 3

20 The purified inhibitor showed inhibitor potency against rat brain AChE with an  $IC_{50}$  of  $15-20 \times 10^{-6}$  M. as assayed according to Ellman et al., (Biochem. Pharmacol. 7(1961), 88-95) and is given as follows: The enzyme inhibition is carried out by pre- incubating the enzyme (rat brain acetylcholinesterase) with 2-20ul of the culture extract or the column fraction at room temperature ( $25^{\circ}$  C) for 15 minutes followed by the addition of the substrate, acetyl  
25 thiocholine iodide (0.5mM), in 3ml phosphate buffer (0.1.M, pH.7.4) containing 0.25mM dithiobisnitrobenzoic acid. Absorbance change at 412 nm is monitored every 30 seconds for 2 min in an UV-VIS Spectrophotometer. Inhibition is calculated relative to the solvent control.  $IC_{50}$  is determined by regression analysis.

#### EXAMPLE – 4

30 Inhibition of serine esterase: The isolated compound (inhibitor) was demonstrated to be an inhibitor of serine esterase as follows:

Inhibition of esterase activity was assayed *in vitro* with the substrate, *p*-nitrophenyl acetate in tris buffer (.05 M, pH 7.4) and the rat liver extract as the source of the enzyme. The

inhibitor, at various concentrations, was pre-incubated with the enzyme in buffer at room temperature for 30 minutes and the residual activity was measured. The product of enzymatic hydrolysis (*p*-nitrophenol) was monitored spectrophotometrically by measuring the absorbance change per minute at 405 nm, according to the method of Sidhu and Blair (J. Biol. Chem., 1975, 250: 7891). Percentage inhibition was calculated with respect to the control (without inhibitor) and IC<sub>50</sub> (the concentration required to inhibit 50% activity) was calculated by regression analysis. IC<sub>50</sub> for the microbial inhibitor was found to be 0.067 μmole/ml which indicates that it is a potent inhibitor of serine esterase.

#### EXAMPLE – 5

Insecticidal properties: The insecticidal property of the inhibitor isolated from *Sporotrichum* sps was investigated as follows:

##### *a. Toxicity to mosquito larvae:*

Late III instar larvae of the mosquito, *Culex quinquefasciatus* (from laboratory culture) were released in four beakers containing tap water (ten larvae in each beaker). The treatment group (2 replicates) was added the inhibitor compound at 100 ppm in 100 μl acetone whereas the control group received acetone only. After 24 hrs, 75 % mortality (7/10 and 8/10) was observed in treated group whereas no mortality was seen in the control group (0/10, 0/10). This experiment demonstrates the insecticidal activity of the compound.

##### *b. Acetyl cholinesterase of the house fly brain:*

Insecticidal potential of the compound was further investigated by the inhibition of the target enzyme, acetylcholinesterase (AChE) from the housefly brain *in vitro*. Ten house fly heads were homogenized in 3 ml buffer (tris .05M, pH 7.4) which served as the source of the enzyme. AChE inhibition was assayed with acetyl thiocholine as the substrate with various concentrations of the inhibitor as described earlier for the rat brain enzyme. Percentage inhibition was calculated with respect to the control (without inhibitor) and IC<sub>50</sub> determined by regression analysis. The compound was found to be a potent inhibitor of the housefly brain AChE with an IC<sub>50</sub> value of 0.98 μmole/ml.

From the above experiment, it is very clear that the compound of the invention is effective in killing or inhibiting the growth of insects, and evidence that the compound functions as an acetylcholinesterase inhibitor. It is well known to those of skill in the art that acetylcholinesterase inhibitors are effective as insecticides.

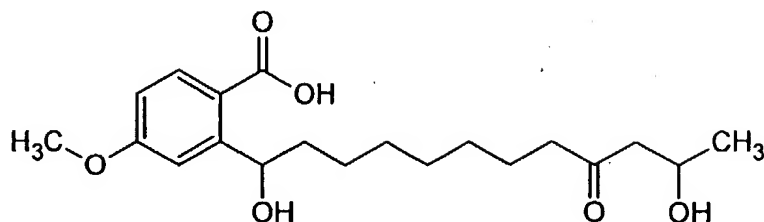


**MAIN ADVANTAGES OF THE PRESENT INVENTION**

1. The present invention provides an AchE /serine esterase/protease inhibitor from a microbial source.
2. The present invention provides a simple extraction and chromatographic procedure to  
5     purify the AchE inhibitor from the crude mixture.
3. In the present invention the isolated inhibitor is a novel bioactive molecule

## Claims

1. A compound having the formula:



5

2. Use of the compound of formula 1 for killing or inhibiting the growth of an insect comprising administering to the insect an effective amount of the compound.
3. Use as claimed in claim 2 wherein said insect is a mosquito.
- 10 4. Use as claimed in claim 3 wherein said mosquito is *Culex quinquefasciatus*.
5. The compound according to claim 1 is as an inhibitor of serine esterase.
6. Use of the compound of formula 1 for application as a drug for Alzheimer's disease or dermentia
7. A process for the isolation of the compound according to claim 1, from the fungus
- 15 Sporotrichum species, said process comprising the steps of:
- (a) extracting fermented solid with an organic solvent;
- (b) filtering the extract of step (a) through a cloth or Whatman filter paper to obtain a clear solution;
- (c) evaporating the solution of step (b) under reduced pressure to obtain a crude
- 20 extract;
- (d) purifying the crude extract of step (c) by column chromatography over silica gel and eluting with mixture of organic solvents of increasing polarity;
- (e) pooling active eluted fractions of step (d) and further subjected to column chromatography over silica gel by eluting with a mixture of organic solvents with increasing polarity;
- 25 (f) repooling the active eluted fractions of step (e);
- (g) evaporating the pooled fractions of step (f) to yield the pure compound of formula I and
- (h) dissolving the residue in step (g) in ethyl acetate to yield the pure compound of
- 30 claim 1.

8. The process according to claim 7, wherein in step (a) the organic solvent is selected from the group consisting of ethyl acetate, acetone and methanol.
9. The process according to claim 7, wherein in step (d) the mixture of organic solvents is selected from the combination of hexane: diethyl ether and chloroform: methanol mixtures.
- 5 10. The process according to claim 7, wherein in step (e) the mixture of organic solvent used is chloroform: ethyl acetate mixture.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IN 03/00073

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C07C65/40 A61K31/192

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

CHEM ABS Data, WPI Data, EPO-Internal, MEDLINE, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	OMURA, SATOSHI ET AL: "Arisugacin, a novel and selective inhibitor of acetylcholinesterase from <i>Penicillium</i> sp. FO-4259" JOURNAL OF ANTIBIOTICS , vol. 48, no. 7, 1995, pages 745-746, XP009013727 cited in the application the whole document	1

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*A\* document member of the same patent family

Date of the actual completion of the international search

15 July 2003

Date of mailing of the international search report

06/08/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Goetz, G

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IN 03/00073

### Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claim 6 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.